

Prevalence of Angiotensin II Type 1 Receptor Antibodies in Persons with Hypertension and Relation to Blood Pressure and Medication

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ABSTRACT

Background: We aimed to determine the prevalence of antibodies against angiotensin II type 1 receptor (AT1Rabs) in hypertensive adults and elucidate the relation of anti-hypertensive medication type to blood pressure (BP) among persons with and without AT1Rab.

Methods: Sera from participants in the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study with hypertension were tested for AT1Rab using a commercial ELISA [(OneLambda; positive ≥ 17 units/ml)]. BP measurements, uncontrolled BP (systolic BP ≥ 140 and/or diastolic BP ≥ 90 mmHg) and effect of BP medication type were compared for AT1Rab positive (+) versus negative (-) participants using descriptive statistics and multivariable regression.

Results: 132 (13.1%) participants were AT1Rab+. Compared to AT1Rab-, AT1Rab+ persons were more likely to be white (47 vs. 36.7%; $p = 0.03$) but had similar comorbid disease burden. In models adjusting for age, sex, and race, AT1Rab+ persons had higher diastolic BP ($\beta = 2.61$ mmHg; $SE = 1.03$; $p = 0.01$) compared to AT1Rab- participants. Rates of uncontrolled BP were similar between the groups. AT1Rab+ persons on an ARB ($n = 21$) had a mean of 10.5 mmHg higher systolic BP ($SE = 4.56$; $p = 0.02$) compared to AT1Rab+ persons using other BP medications. The odds of uncontrolled BP among AT1Rab+ participants on an ARB was 2.05 times that of those on other medications. AT1Rab- persons prescribed an ACEi had 1.8 mmHg lower diastolic BP ($SE = 0.81$; $p = 0.03$) than AT1Rab- persons not prescribed an ACEi.

Conclusion: AT1Rab was prevalent among hypertensive adults and was associated with higher BP among persons on an ARB.

Keywords: angiotensin II type 1 receptor antibody, hypertension, blood pressure, health disparities

Introduction

The renin and angiotensin system (RAS) has been the target of several therapeutic drugs designed to regulate blood pressure (BP)¹⁻³. However, evidence shows variability in the efficacy of these therapies based on patient characteristics⁴⁻⁷. Within RAS, angiotensin II and the angiotensin receptors are central to maintenance of blood pressure control^{8,9}. Under normal conditions, cellular homeostasis is maintained by alternate vasoconstriction and vasodilation of the arteries as angiotensin II binds to its receptors, angiotensin II type 1 (AT1R) and type 2 (AT2R), respectively. When angiotensin II binds to AT1R, in addition to vasoconstriction, it mediates other important physiological functions such salt and water retention, aldosterone secretion and vascular remodeling¹. Under stressful conditions that lead to loss of homeostasis, a more persistent or recurrent activation of AT1R by its ligand, angiotensin II, results in extended vascular constriction, inflammation and development of fibrosis; all of which contribute to dysregulation of the vascular endothelium¹⁰.

Similar to angiotensin II, antibodies directed against the angiotensin II type 1 receptor (AT1RAb) activate the receptor, AT1R. AT1RAbs have been detected in the sera of patients with conditions associated with development of hypertension^{11,12}, such as preeclampsia¹³, and detected in kidney, heart, and liver transplant recipients with vascular rejection¹⁴⁻¹⁸. Treatment protocols for patients who develop allograft dysfunction following organ transplantation in the presence of AT1RAb are not well defined. A few single center studies have reported the use of angiotensin receptor blockers (ARB), which target the AT1 receptor, as a mechanism to prevent allograft rejection^{19,20}. The effect of these and other anti-hypertensive medications on BP in the presence of AT1RAb is not known. In this study, we aimed to determine the prevalence of AT1RAb in community-dwelling hypertensive adults and elucidate the relationship between presence of AT1RAb and medication-specific BP regulation.

Methods

Study Population

We performed a cross-sectional analysis of data from the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. HANDLS is a population-based cohort study examining the influence and interaction of race and socioeconomic status on the development of cardiovascular and cerebrovascular health disparities among minorities and lower socioeconomic status groups²¹. Participants are black and white community-dwelling individuals between the ages of 30 and 64 at enrollment who are from 13 neighborhoods (group of contiguous census tracts) in both low and high socioeconomic strata in Baltimore City, Maryland. Participant enrollment took place from August 2004 to November 2009, and follow-up evaluations are completed every 5 years. Each participant provided written informed consent. The protocol was approved by the National Institute of Environmental Health Sciences, National Institutes of Health. In the present study, 1006 HANDLS participants with self-reported hypertension at study baseline were included, and their sera was examined for detection of antibodies against AT1R. Blood pressure measurements collected from follow up visits were compared to antibody levels.

Measurements

Stored sera with unique identifiers were shipped on ice from repositories located at the National Institute on Aging to the Johns Hopkins Immunogenetics Laboratory, stored at -80°C , and tested in batches over a period of 4 months. Detection of AT1RAb was performed using a validated quantitative ELISA (One Lambda, ThermoFisher). The samples were run in duplicate on microtiter plates coated with human AT1R derived from transfected Chinese hamster ovary cell extracts. Duplicate results with greater than 20% CV were repeated. Presence of antibody bound to AT1R was detected by a colorimetric change. Optical density values were converted into a concentration expressed as unit per milliliter (units/ml) based on a standard curve generated using control samples at varying concentrations (2.5, 5, 10, 20 and >40 units/ml). AT1RAb was reported as positive for concentrations ≥ 17 units/ml according to manufacturer recommendations as well as previous reports from the Immunogenetics Laboratory at Johns Hopkins²².

BP and uncontrolled BP were our primary outcomes of interest. Each participant underwent sitting and standing blood pressure measurements on each arm using the brachial artery auscultation method with an inflatable cuff of appropriate size²³. We examined the relation of AT1RAb positivity to (a) systolic and diastolic BP (average of sitting and standing measures); (b) uncontrolled BP defined as an average baseline systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg; and (c) uncontrolled BP by anti-hypertensive medication type. Medications were categorized by review of participants' self-reported medication lists. Other factors that may contribute to differences in AT1RAb status and/or blood pressure were examined, including participants' age, sex, race, body mass index²⁴, history of smoking, evidence of inflammation^{25,26} and comorbid conditions such as diabetes^{8,27} and cardiovascular disease²⁸. Age, sex, race (African American or White) and household income level were self-reported at the doorstep during household enrollment in HANDLS. The presence of relevant comorbid diseases was ascertained using medical history, physical examination, and laboratory assessment on a mobile research vehicle (MRV). Health behaviors such as tobacco use were self-reported. Diabetes mellitus was defined as fasting plasma glucose concentration ≥ 126 mg/dL (7.0 mmol/L), self-report of diabetes or use of hypoglycemic agents. Anthropometric measurements were performed, including height and weight, and were used to calculate body mass index. Fasting venous blood was collected on the MRV. High sensitivity C-reactive protein levels were measured from blood samples by immunoassay at the National Institute on Aging or Quest Diagnostics using similar equipment and reagents²⁹.

Statistical Analysis

Differences in participant characteristics by AT1RAb status were examined using Fisher's exact tests for categorical variables and t-tests or Wilcoxon–Mann–Whitney test for continuous variables. Unadjusted and multivariable logistic regression models were developed to estimate the association between participant characteristics and AT1RAb status. The association between participant AT1RAb status and BP was examined using t-tests and linear regression models adjusting for demographic characteristics (age, sex, and race). The association between AT1RAb status and uncontrolled BP was examined using Fisher's exact tests and logistic regression adjusting for

demographic characteristics. Linear regression models were developed to estimate the association between medication use and blood pressure among AT1RAb positive and negative participants, adjusting for demographic characteristics. The association between medication use and uncontrolled BP among AT1RAb positive and negative participants was examined using logistic regression. Alpha (two-tailed) was set at $<.05$ and data were analyzed using Stata 15.1 (StataCorp LLC, Texas, 2017).

Results

Participant Characteristics Overall and by AT1RAb Status

Of 3,720 participants enrolled in the HANDLS study, we identified 1006 with self-reported hypertension who had available serum for AT1RAb testing. Participant characteristics are described in **Table 1**. The cohort consisted of 59% women, 62% African Americans, with a mean age at enrollment of 52 years. Overall, 132 participants (13.1%) were positive for AT1RAb (**Table 1**). AT1RAb+ and AT1RAb- participants were of similar age and had similar sex composition, however, compared to AT1RAb- participants, a greater proportion of AT1RAb+ participants were White (47% versus 36.7%; $p = 0.03$); 16% (62 of 383) of White participants were AT1RAb+ while 11% of African American participants (70 of 623) were AT1RAb+. Former or current history of smoking tobacco was more common among AT1RAb- participants (70.4%) as compared to AT1RAb+ participants (56.2%); $p=0.002$ (**Table 1**). In multivariable logistic regression models, White race and tobacco use had independent associations with AT1RAb status. White race was associated with greater odds of being AT1RAb+ [Odds ratio (OR) 1.56, 95% Confidence Interval (CI) 1.05-2.31] and tobacco use was associated with lesser odds of being AT1RAb+ (OR 0.52, 95% CI 0.35-0.78) (**Table 2**). No other demographic or clinical characteristics were statistically significantly associated with AT1RAb status.

Blood Pressure by AT1RAb Status and Anti-Hypertensive Medication

Mean systolic BP was similar between AT1RAb+ and AT1RAb- participants (127 mmHg versus 126.3 mmHg respectively; $p=0.71$); as was the prevalence of uncontrolled BP across the two groups (33.3% and 28.1%, respectively; $p=0.25$) (**Table 3**). Conversely, the mean diastolic BP in the AT1RAb+ group was statistically significantly higher than in the AT1RAb- group (77.3 mmHg

versus 75 mmHg; $p = 0.03$). In multivariable regression models, these relationships were similar, with a statistically significantly higher diastolic BP among AT1Rab+ participants ($p=0.01$) (**Table 4**). The adjusted odds ratio (aOR) for uncontrolled BP was 1.33 (95% CI 0.89-1.98) for AT1Rab+ versus AT1Rab- persons.

A majority (88%) of participants reported being prescribed anti-hypertensive medications, which included angiotensin receptor blockers (ARB), angiotensin converting enzyme inhibitors (ACEi), calcium channel blockers (CCB), alpha and beta-blockers, and diuretics (full list provided in **Supplemental Table 1**). ARBs prescribed included irebesartan, valsartan, losartan, and olmesartan. Most participants were prescribed more than one anti-hypertensive medication, with diuretics (49.8%) followed by ACEi (36.3%) being the most common. ARB prescriptions were noted for 16.3% (143 of 880) of participants. There was no statistically significant difference in medication-types prescribed to participants by AT1Rab status (**Supplemental Table 2**).

In multivariable linear regression analyses, and compared to AT1Rab+ persons who were on other anti-hypertensive medications, AT1Rab+ persons who had been prescribed an ARB had 10.5 mmHg higher SBP ($p=0.02$) and no statistically significant difference in DBP (mean difference 4.7 mmHg; $p = 0.2$) (**Table 5**). There was a trend towards greater odds of uncontrolled BP among AT1Rab+ participants prescribed an ARB compared to those on other anti-hypertensive medications (aOR 2.05, 95% CI 0.76-5.53). In contrast, there was no difference in systolic or diastolic BP among AT1Rab- participants by ARB use (**Table 5**).

AT1Rab+ participants prescribed an ACEi had an adjusted mean of 2.83 higher systolic BP than AT1Rab+ persons not prescribed an ACEi ($p= 0.46$) (**Table 5**). AT1Rab- participants prescribed an ACEi had similar systolic BP but 1.8 mmHg lower diastolic BP than AT1Rab- persons not prescribed an ACEi ($p=0.03$). Participant characteristics by ARB, ACEi and all other medication use is detailed in **Supplemental Table 3**.

Discussion

Among community-dwelling adults with hypertension, we found that the prevalence of AT1RAbs was 13.1%. AT1RAb+ individuals were more likely to be White and had higher diastolic BP than those who were AT1RAb-. AT1RAb+ persons who reported taking an angiotensin receptor blocker had higher systolic BP than antibody positive individuals taking other antihypertensive medications. Among participants who were AT1RAb-, BP did not differ by ARB use, however, those prescribed an ACEi had modestly lower diastolic BP than persons not prescribed an ACEi.

Antibodies against AT1R have been identified that bind to a specific sequence within the second extracellular loop (ECL2) of the receptor^{14,30}. When bound to the ECL2, these autoantibodies were shown to cause activation of signaling through phosphorylation of the extracellular signal-regulated kinase (ERK) pathway²⁰ which results in upregulation of pro-inflammatory targets³¹, endothelial cell damage and BP dysregulation. In a sample of 315 older adults, with a different ELISA which uses purified capture antigen, AFHYESQ (one of the 2 sequences required for antibody binding as was described by Dragun et al²⁰), higher AT1RAb concentrations were associated with hypertension, inflammation, and frailty²⁴. AT1RAb concentrations in the same study were higher among individuals over 70 years old than among younger individuals. Here, we report, in a larger and younger population (median age of 52 years) with hypertension, that there was a trend towards AT1RAb+ individuals being more likely to have uncontrolled BP as compared to AT1RAb- persons (33% versus 28%, respectively). Mikolajczyk et al, recently summarized the evidence for the role of the adaptive immune response and hypertension¹².

In our analyses by antihypertensive medication type, we noted variation in BP for AT1RAb+ participants depending upon whether they were treated with an ARB or another antihypertensive. Many of the angiotensin receptor blockers target residues within the ECL2 region of AT1R and block the activation of the receptor³². The effect of AT1RAb on arterial contraction has been illustrated in animal models, as has the response to ARBs. AT1RAbs cause increased arterial contractility in rats, which can be blocked with losartan³³. Abadir et al further showed that treatment with ARBs was associated with better control of systolic BP in patients with higher AT1RAb levels compared to patients with low AT1RAb²⁴. Taking this existing literature together, we expected to find that

AT1RAb+ persons in our study who were prescribed an ARB would have *better* BP control, however we found the converse. Notably, studies that have evaluated the interaction between the AT1R and a number of angiotensin receptor blockers, have found differences in the ability of individual ARBs to effectively inactivate AT1R^{18,34}. For example, candesartan has been reported to be more effective compared to losartan in treatment of patients with vascular-mediated solid organ transplant rejection because of higher AT1R avidity and half-life³⁴. Furthermore, mutant models of AT1R that resemble the active conformational structure of this receptor, showed that epitopes that are targeted by losartan become inaccessible in a constitutively active form of AT1R compared to a receptor that alternates between resting and active state³⁴. With a modest number of AT1RAb+ participants prescribed an ARB (n=143), we were unable to explore the effects of different ARBs on BP, thus larger studies of this subject are warranted and could have important therapeutic implications.

Interestingly, a few studies have shown that AT1RAb may also affect intracellular calcium concentrations independent of the known target receptor, AT1R^{35,36}. Wang et al³⁵ show that in BALB/C mice injected with AT1RAb and exhibiting phenotypes similar to preeclampsia (ie development of hypertension and proteinuria), there was a decrease in the expression of potassium calcium channels which are important for regulation of intracellular Ca²⁺ concentration. The use of valsartan, an ARB, did not show improvement in the phenotypes of these preeclamptic animals. Yu et al³⁶ further showed that AT1RAb isolated from patients with preeclampsia and incubated in a preeclampsia podocyte injury rat model resulted in a dose dependent induced podocyte injury and changes in intracellular Ca²⁺ concentration³⁶. Of note, in our study, when participants expressed AT1RAb, BP control was better among those prescribed other medications as compared to those prescribed an ARB.

The characteristics of participants on an ARB were similar to those on other medications, with slightly higher BMI and more females among those reporting ARB use. Many of these antihypertensive drugs target pathways that are independent of RAS (calcium channel blockers, beta blockers) or work upstream of the AT1R. For example, angiotensin converting enzyme inhibitors prevent formation of the natural ligand angiotensin II. Recent studies have shown improved outcomes in the treatment for hypertension with use of new combination drugs³⁷. Future studies are needed that

are inclusive of adequate numbers of participants with and without AT1RAb and treated with a diversity of antihypertensives medications in order to advance understanding of the appropriate treatment for hypertension in the setting of AT1RAbs.

In our study, White race was a predictor of AT1RAb positivity. Whether this is due to genetic ancestry more common among individuals who self-identify as White is unknown. Allele variants of AT1R have been identified and these variants are associated with variable increase in receptor expression³⁸⁻⁴⁰. It is possible that during an inflammatory event that causes endothelial cell damage, individuals with higher AT1R expression would present autoantigens and develop autoantibodies. Further work is needed to assess the role of allele expression based on genetic ancestry in this process.

Our study had limitations, including (a) its cross-sectional design, (b) the limited number of AT1RAb+ participants taking ARBs and other antihypertensive medications to allow for a more detailed analysis across medication types, and (c) the lack of a measure of adherence to reported medications. We employed a commonly used and validated commercial assay to measure AT1RAbs based on immunoreactivity to full length AT1R. The Johns Hopkins Immunogenetics Laboratory, in collaboration with 10 other laboratories through proficiency testing has established the rate of concordance as 90% with the 10% discordant results due to variations between the negative versus borderline cutoffs as determined by the manufacturer of the assay (10-17 Units/ml is considered borderline). The assay measures the quantity of the autoantibodies but does not address their biological activity. Work by Dragun et al and other investigative teams have described an agonistic activity to AT1RAbs based on using enriched IgG fractions from patients positive for AT1RAbs in either competition assays, cell based readouts in the presence or absence of ARBs, or for injection into mice^{19,20,31,35}. Therefore, further basic research is required to map out the mechanism of action of these antibodies.

Strengths of our study include it being, to our knowledge, the first to investigate the prevalence of AT1RAb in community-dwelling hypertensive individuals. We were also able to explore the relation of medication type to BP control by AT1RAb status which can serve to generate hypotheses for future work in this area.

In conclusion, we found AT1RABs to be prevalent among a sample of individuals with hypertension, and also found that BP control among AT1RAB+ persons varied by medication type. Towards greater precision in hypertension treatment, we identified an immunologic factor that may affect BP regulation in a subset of individuals and is worthy of further study.

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Table 1: Demographic and Clinical Characteristics Overall, and by AT1Rab status

Patient Characteristics	N for analysis	Overall	AT1Rab positive	AT1Rab negative	p-value
AT1Rab status, n (%)	1006	--	132 (13.1)	874(86.9)	--
Age, mean (SD)	1006	52.4 (8.4)	52.5 (8)	52.4 (8.5)	0.86
Male, n (%)	1006	408 (41)	57 (43.2)	351 (40.2)	0.51
White race, n (%)	1006	383 (38.1)	62 (47)	321 (36.7)	0.03
Income below poverty level, n (%)	1006	426 (42.4)	57 (43.2)	369 (42.2)	0.85
Former and/or current history of smoking, n (%)	991	679 (68.5)	73 (56.2)	606 (70.4)	0.002
Body mass index [kg/m ² , mean (SD)]	1006	32.4 (8.1)	31.9 (8.1)	32.5 (8.1)	0.42
Liver cirrhosis, n (%)	1002	9 (0.9)	3 (2.3)	6 (0.7)	0.1
Congestive heart failure, n (%)	996	62 (6.2)	9 (6.9)	53 (6.1)	0.7
Coronary artery disease, n (%)	996	81 (8.1)	8 (6.1)	73 (8.4)	0.49
Atrial fibrillation, n (%)	995	131 (13.1)	21 (16)	110 (12.7)	0.33
Diabetes, n (%)	989	299 (30.2)	36 (27.9)	263 (30.6)	0.61
C-reactive protein, median (p25-p75)	982	3 (1.2-7.3)	3.5 (1.5-7.8)	2.9 (1.1-7.1)	0.15
AT1Rab concentration [units/ml, mean (SD)]	1006	4.7 to >40	>28.3 (9.6)*	9.4 (2.8)	--

* Within the AT1Rab+ group, 44 participants had very high AT1Rab concentrations with values that exceeded the maximum limit of detection of the ELISA; these concentrations were reported as >40 units/ml.

Table 2: Multivariable logistic regression of variables with AT1Rab status

Variable	Odds ratio comparing AT1Rab Positive to Negative Participants	95% Confidence Interval
Age	1.005	0.98, 1.03
Male	1.14	0.76, 1.7
White race	1.56	1.05, 2.31
Income below poverty level	1.14	0.76, 1.7
Former and/or current history of smoking	0.52	0.35, 0.78
Body mass index (kg/m ²)	0.99	0.96, 1.01
Liver cirrhosis	2.84	0.67, 11.98
Congestive heart failure	1.22	0.56, 2.65
Coronary artery disease	0.71	0.32, 1.55
Atrial fibrillation	1.31	0.75, 2.28
Diabetes	0.86	0.55, 1.34
C-reactive protein	1.01	0.99, 1.02

*All variables (covariates) included.

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Table 3 Absolute BP and BP control by AT1RAb status

Variable	N	Overall I	AT1RAb positive	AT1RAb negative	p- value
Systolic BP (mmHg), mean (SD)	98 6	126.4 (18.9)	127 (18.8)	126.3 (19)	0.71 ¹
Diastolic BP (mmHg), mean (SD)	98 5	75.3 (11.1)	77.3 (11.2)	75 (11.1)	0.03 ¹
Uncontrolled BP, n (%)	98 6	284 (28.8)	43 (33.3)	241 (28.1)	0.25 ²

¹t-test; ²Fisher's exact test

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Table 4: Multivariable Analysis of BP measurements by AT1RAb Status (Positive versus Negative), Adjusted for age, sex and race

Variable	N	Beta(SE) or OR(95%CI)	p-value
Systolic BP (mmHg)	986	1.14 (1.75)	0.52 ³
Diastolic BP (mmHg)	985	2.61 (1.03)	0.01 ³
Uncontrolled BP	986	1.33 (0.89, 1.98)	0.17 ⁴

³Linear regression; ⁴Logistic regression. Both linear and logistic regression adjusted age, sex and race
Uncontrolled BP: Systolic BP \geq 140 mmHg and/or Diastolic BP \geq 90 mmHg.

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Table 5: Association between prescribed anti-hypertensive medication and BP by AT1Rab status

AT1Rab positive					
Predictor	N¹	Outcome	Beta (SE)²	p-value	Uncontrolled BP (OR, 95% CI)
ARB use	115	SBP	10.52 (4.56)	0.02	2.05 (0.76, 5.53)
	115	DBP	4.74 (2.71)	0.21	
ACEi use	115	SBP	2.83 (3.84)	0.46	0.75 (0.31, 1.78)
	115	DBP	0.17 (2.27)	0.94	
AT1Rab negative					
Predictor	N	Outcome	Beta (SE)	p-value	Uncontrolled BP (OR, 95% CI)
ARB use	745	SBP	0.58 (1.84)	0.75	0.97 (0.62, 1.52)
	744	DBP	1.05 (1.07)	0.33	
ACEi use	746	SBP	-1.24 (1.39)	0.37	0.98 (0.69, 1.38)
	745	DBP	-1.82 (0.81)	0.03	

Linear regression models adjusted for age, sex and race.

Abbreviations: SBP, systolic blood pressure. DBP, diastolic blood pressure. ARB, angiotension receptor blocker. ACEi, angiotensin converting enzyme inhibitor.

¹N: Number of outcomes (SBP, DBP) with ARB/ACEi by AT1Rab status

² SE: Standard error

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